Food Packaging and Shelf Life 31 (2022) 100771

Contents lists available at ScienceDirect

Food Packaging and Shelf Life

journal homepage: www.elsevier.com/locate/fpsl

Application of palladium-based oxygen scavenger to extend the mould free shelf life of bakery products

Nadine Rüegg, Bettina Röcker, Selçuk Yildirim *

Zurich University of Applied Sciences, Institute of Food and Beverage Innovation, Wädenswil, Switzerland

ARTICLE INFO

Keywords:
Oxygen scavenger
Palladium
Active packaging
Gluten-free bread, toast bread
Bread

ABSTRACT

An oxygen scavenging film based on a catalytic system with palladium (CSP) was combined with modified atmosphere (MA) packaging to extend the mould free shelf life (MFSL) of bakery products. Par-baked buns, toast bread and gluten-free bread inoculated with *Aspergillus niger* spores were packed in normal atmosphere (NA) and under MA (with 2 vol.-% of O_2) with or without CSP. Mould growth was detected after 2–3 days on all products packed under NA as well as under MA without CO_2 and CSP. Use of CO_2 in MA extended the MFSL by 8–10 days, 16–18 days and 3–4 days for par-baked buns, toast and gluten-free bread, respectively. Use of CSP with MA reduced the oxygen concentration in headspace from 2 vol.-% to < 0.01 vol.-% within 105–190 min with all bakery products. This led to a further increase in MFSL of bakery products by 3–9 days.

1. Introduction

Shelf life of bakery products like bread and buns is mainly limited by staling and microbial growth. Although staling is the main limiting factor for bread, microbial growth becomes more important when the bread is packed. Every bakery product has a specific shelf life, which depends on the type of ingredients used and the environment in which it is packaged (Axel, Zannini, & Arendt, 2017). Without the use of antimicrobial preservatives and additional packaging technologies (e.g. modified atmosphere packaging or clean room technology), bread can be expected to have a shelf life of 2–3 days (Suhr & Nielsen, 2005). It has been shown that the microbial spoilage of bakery products is mostly attributed to moulds like *Penicillium spp*. and *Aspergillus niger* (Legan & Voysey, 1991). Apart from the repelling appearance of visible growth, moulds are also responsible for off-flavour formation and the production of mycotoxins and allergenic compounds. Visible yeast growth, commonly known as chalk moulds, are also important spoilers of bread (Deák, 2007; Legan & Voysey, 1991; Nielsen & Rios, 2000; Smith, Daifas, El-Khoury, Koukoutsis, & El-Khoury, 2004). Contamination of bread with moulds and yeasts mainly occurs during cooling and subsequent packaging (Galić, Curić, & Gabrić, 2009). To retard microbial spoilage in bakery products, antimicrobial preservatives including organic acids (propionic, benzoic, sorbic, acetic and their salts) and ethanol is used (Smith et al., 2004).

Today, consumers demand bakery products with a longer shelf life but without any synthetic preservatives (Axel et al., 2017). To achieve this, modified atmosphere (MA) packaging mainly with a combination of N_2 and CO_2 is used. On one hand CO_2 in MA has an antimicrobial effect and can help to retard the microbial growth (Church, 1994). On the other hand, MA packaging can help to reduce the oxygen in the headspace, which is necessary for the growth of aerobic microorganisms in bread. However, on industrial scale MA packaging may not be able to reduce the headspace oxygen concentrations to very low levels due to insufficient evacuation as well as due to the fact that oxygen is trapped in the bakery products may release subsequently into the headspace after packaging (Yildirim et al., 2018). Typically, 0.5 – 5 vol.-% of oxygen remains in the headspace which still enables mould growth (Haasum & Nielsen, 1998; Tabak & Cooke, 1968). Nielsen and Rios (2000) and Suhr and Nielsen (2005) found that typical bakery moulds like *Penicillium commune, P. roqueforti* and *Aspergillus flavus* were able to grow even at residual oxygen levels of 0.03% depending on the CO_2 content used in the MA. It was also shown that *Aspergillus spp*. has a greater sensitivity to the effects of CO_2 then *Penicillium spp*. (Rodriguez, Medina, & Jordano, 2000; Seiler, 1989).

Innovative packaging technologies, such as oxygen scavengers, can remove residual oxygen in the headspace and thereby prolong the shelf life of bakery products and eliminate the need of antimicrobial preservatives (Upasen & Wattanachai, 2018). Most commonly used oxygen scavengers are iron-based systems in the form of sachets (Dey & Neogi, 2019; Vilela et al., 2018). However, the application of this type of

* Correspondence to: Zurich University of Applied Sciences, Institute of Food and Beverage Innovation, Einsiedlerstrasse 34, 8820 Wädenswil, Switzerland.
E-mail address: selcuk.yildirim@zhaw.ch (S. Yildirim).

https://doi.org/10.1016/j.fpsl.2021.100771
Received 18 May 2021; Received in revised form 27 September 2021; Accepted 5 November 2021
Available online 25 November 2021

scavenger is limited due to limited consumer acceptance of sachets in food packages and the requirement of an additional packaging operation step (Gibis & Rieblinger, 2011; Pereira de Abreu, Cruz, & Paseiro Losada, 2012; Restuccia et al., 2010; Vilela et al., 2018). Recently, an oxygen scavenging film based on a catalytic system with palladium (CSP) was developed, which can remove up to 2.5 vol.-% residual oxygen in the headspace very quickly (Röcker, Rüegg, Glöss, Yeretzian, & Yildirim, 2017; Yildirim, Röcker, Rüegg, & Lohwasser, 2015). Palladium catalyses the oxidation of hydrogen into water (Nyberg & Tengstål, 1984; Wanner, 2010), and thus can remove the residual headspace oxygen of a modified atmosphere packaging containing hydrogen (Lohwasser & Wanner, 2005; Sängerlaub, Witzgall, Müller, Wiegert, & Pecyna, 2021; Yildirim, Jammet, & Lohwasser, 2010). CSP has been successfully applied into ham packaging to prevent colour change in ham (Hutter, Rüegg, & Yildirim, 2016) and lipid oxidation in linseed oil (Faas, Röcker, Smrke, Yeretzian, & Yildirim, 2020).

In this study, the potential application of the developed catalytic system based on palladium for the removal of oxygen in the headspace and its influence on mould growth on par-baked buns, toast bread slices and gluten-free bread slices has been studied. For that purpose, bakery products were packed with CSP under modified atmosphere containing 2 vol.-% oxygen (to simulate residual oxygen) with and without CO_2 and stored at 25 °C. During storage, oxygen concentration in the headspace was monitored and mould growth was visually observed.

2. Materials and methods

2.1. Catalytic system based on palladium (CSP)

Palladium (1.2 nm) was vacuum-deposited on a PET/SiOx film (38.5 μm/ 89.9 nm) using magnetron sputtering technology as previously described by Yildirim et al. (2015). The CSP was cut into labels (25 cm^2) and one label per package was attached by a scotch tape to the inner side of the lidding film (57 μm PET/PE-EVOH-PE, Südpack Verpackungen, Germany). The active side of the CSP faced towards the headspace of the packaging without being in direct contact with the packed food.

2.2. Preparation of spore suspension

Aspergillus niger ATCC 16404 was grown on malt agar plates composed of 1.8% malt extract (Biolife, Italy) and 1.8% agar (Biolife, Italy) for 12 days at 25 °C in the dark to obtain sporulation. For harvesting, 5 ml sterile buffered peptone water (0.15%. Merck, Germany) were poured into each plate. The spores were carefully detached and removed using a pipette (Eppendorf AG, Germany). Subsequently, the spore suspension was diluted with sterile glycerol (87%, AppliChem GmbH, Germany) in a ratio of 1:1 and stored in 2 ml Eppendorf tubes (tree AG, Switzerland) at − 20 °C until usage. Prior to usage, the spore concentration in the spore suspension was determined by a cultural method. The calculated spore concentration was used to set the target concentration of 10^5 (spores/ml) for the inoculation process.

2.3. Preparation of par-baked buns, toast and gluten-free bread

Par-baked buns were produced with wheat flour (type 550, Willi Grüninger, Switzerland). The other ingredients on flour basis, were 72% tap water, 3% margarine (Bettina LT, Pistor, Switzerland), 4% backing agent (Eismalz, Pistor, Switzerland), 2% salt (JuraSel, Pistor, Switzerland) and 3% yeast (Pistor, Switzerland). All ingredients were mixed for 480 s at 20 Hz, 300 s at 50 Hz and afterwards 120 s at 40 Hz with lab kneader (Diosna SP4). After 5 min bulk fermentation at room temperature, the dough was mechanically divided into 2100 g portions and proofed for 20 min at room temperature. Afterwards the dough was divided into 60 g portions, mechanically moulded (Quadro-King 2000, Huwiler Technics, Switzerland), 30 min proofed at 20 °C and 75% rel. humidity, stamped and turned and afterwards again proofed for 120 min at 30 °C and 75% rel. humidity. Then the dough buns were turned again and baked for 5 min at 200 °C and 10 min at 180 °C in a rack oven (MIWE, Germany). After baking, the par-baked buns were cooled at room temperature, packed under normal atmosphere into high barrier PET/AlOx/PE bags (Wipf AG, Switzerland) and frozen at − 16 °C until use.

Toast bread was produced with wheat flour (type 550, Willi Grüninger, Switzerland). The other ingredients, on flour basis, were 55% tap water, 1% backing agent (Alpaka Soft, Puratos, Switzerland), 2% salt (JuraSel, Pistor, Switzerland), 2% rapeseed oil (Pistor, Switzerland) and 4% yeast (Pistor, Switzerland). All ingredients were mixed for 300 s at 20 Hz, 240 s at 40 Hz, 300 s at 45 Hz and 120 s at 50 Hz with the lab kneader (Diosna SP4). After 10 min, bulk fermentation at 25 °C the dough was divided into 2812 g portions and proofed for 10 min at 25 °C. 750 g dough was filled into the toast bread tin (300 × 90 × 90 mm) and closed with lid. Proofing was performed at 30 °C and 75% rel. humidity. Then the toast breads were baked for 20 min at 230 °C, 15 min at 220 °C and 15 min at 200 °C in multi-deck oven (MIWE, Germany). After cooling at room temperature, the toast breads were packed under normal atmosphere into high barrier PET/AlOx/PE bags (Wipf AG, Switzerland) and frozen at − 16 °C until use.

Par-baked gluten-free bread without preservatives (250 g per portion) was purchased from a German industrial bakery (Rustico + Amaranth, Schnitzer, Germany).

2.4. Packaging and storage of bakery products

24 h before packaging, par-baked buns and toast bread were defrosted at 5 °C. 2 par-baked buns were packed per sample. Defrosted toast breads were sliced (Agiloline B100, Bizerba, Germany) at room temperature to a thickness of 15 mm and 1 slice per sample was placed in each package. Gluten-free bread samples were ready baked for 15 min at 200 °C on the day of packaging. After cooling at room temperature, the bread was sliced to a thickness of 15 mm and 1 slice per sample was placed in each package. Packages consisted of a PS-EVOH-PE tray (thickness: 0.5 mm, packaging volume: 550 cm^3 for toast bread slices and gluten-free bread slices and 1200 cm^3 for par-baked buns, Stäger & Co AG, Switzerland) and a 57 μm PET/PE-EVOH-PE lidding film (surface area: 0.024 m^2, O_2 transmission rate ≤2.5 $cm^3/(m^2 * d * bar)$ at 23 °C and 50% relative humidity; Südpack Verpackungen, Germany). The oxygen transmission rate of the whole package (tray and lidding film) was calculated as 1.6–1.7 $cm^3/(m^2 * d * bar)$ based on the oxygen ingress in empty packages. This is comparable to the oxygen transmission rate of packages containing EVOH based trays (Pettersen, Gällstedt, & Eie, 2004). The resulting headspace volume was ~410 cm^3 for par-baked bun samples, ~460 cm^3 for toast bread slices and ~490 cm^3 for gluten-free bread slices. This results in product to headspace ratios for par-baked buns, toast bread and gluten-free bread of 1/0.5 [v/v], 1/4 [v/v] and 1/5 [v/v], respectively. To measure the headspace oxygen concentration, an oxygen sensitive sensor spot (PST3 for normal atmosphere (NA)) and PST6 for modified atmosphere (MAP) with 3 mm diameter (PreSens, Germany) was glued to the inner side of the lidding film prior to sealing. 10 samples each were packed with normal atmosphere (NA, ~20.95 vol.-% O_2) or two different modified atmospheres (2 vol.-% O_2, 5 vol.-% H_2, 93 vol.-% N_2 or 2 vol.-% O_2, 5 vol.-% H_2, 93 vol.-% CO_2, Pangas, Switzerland) using a traysealer (vacuum: 30 hPa, gas flushing: 950 hPa, Multivac T200, Multivac Export AG, Switzerland). After packaging, each toast bread slice and gluten free bread slice was inoculated with a syringe through an airtight septum at three points with 0.1 ml of an *Aspergillus niger* spore suspension and each par-baked bun was inoculated at two points to obtain a final concentration of 1×10^4 cfu per inoculation point. An example for the package setting is shown in Fig. 1. Afterwards, all samples were stored at 25 ± 1 °C until visible mould was detected. Headspace oxygen was measured with a non-destructive fibre optic oxygen transmitter (Fibox 4 trace, PreSens GmbH, Germany) (n = 3).

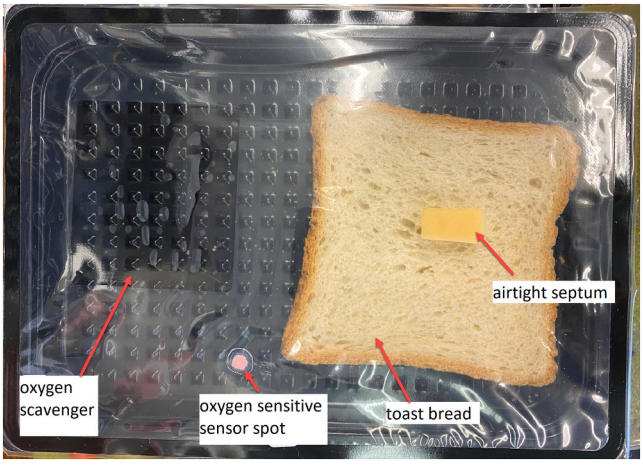

Fig. 1. Experimental set-up to evaluate the influence of palladium-based oxygen scavenger on the mould free shelf life of bakery products. High barrier tray containing the oxygen scavenger (catalytic system based on palladium) attached on the lidding film, toast bread slice, airtight septum through which the bakery sample was inoculated and an oxygen sensitive sensor spot.

3. Results

To study the potential use of an oxygen scavenging film based on a catalytic system with palladium to retard the growth of moulds in bakery products, par-baked buns, toast bread slices and gluten-free bread slices were packed at normal atmosphere or under modified atmosphere with 93 vol.-% N_2 or CO_2, 2 vol.-% O_2 and 5 vol.-% H_2, with or without CSP. During storage at room temperature headspace oxygen was monitored and mould growth was visually detected.

3.1. Evaluation of the oxygen scavenging activity of the catalytic system based on palladium in the headspace

Use of CSP in modified atmosphere (MA) containing CO_2 reduced the initial headspace oxygen concentration of non-inoculated packed bakery products very fast from 2 vol.-% to below 0.01 vol.-% (measurement accuracy) within 190 min for par-baked buns, 115 min for toast bread slices and 105 min for gluten-free bread (Fig. 2). Similar results were obtained for bakery products packed under MA without CO_2 (data not shown). In empty trays, the headspace oxygen was reduced within 40 min for trays used for toast bread and gluten-free bread slices and within 80 min for trays used for par-baked buns. This indicates that the presence of the bakery products reduced the oxygen scavenging activity of the CPS slightly, which is probably due to the presence of volatile sulphur compounds released from the bakery products (Röcker et al., 2017). The time to scavenge all the oxygen in the headspace could have been also prolonged by the diffusion of the trapped oxygen from the bakery products into the headspace.

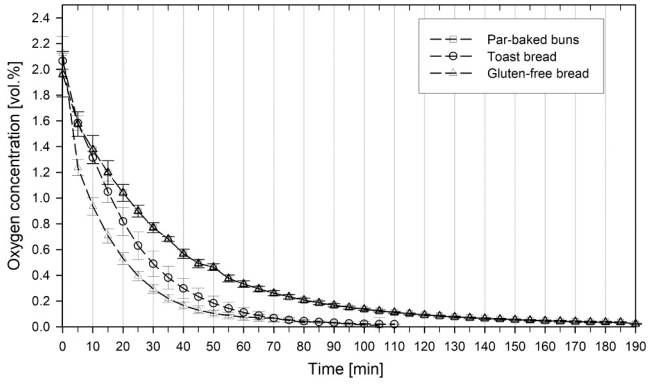

Fig. 2. Reduction in headspace oxygen concentration of par-baked buns, toast bread slices and gluten-free bread slices packed under modified atmosphere (93 vol.-% CO_2, 2 vol.-% O_2 and 5 vol.-% H_2) with CSP and stored at 25 ± 1 °C. Headspace volume (HSV) was for par-baked buns ~410 cm^3, toast bread slices ~460 cm^3 and gluten-free bread slices ~490 cm^3. Mean values ± standard deviation (n = 3).

When oxygen is available in packed bakery packages it takes generally 2–3 days for mould to grow and be visible on the bakery products (without preservatives) (Suhr & Nielsen, 2005). Consequently, in order to prevent mould growth, it is important to remove the oxygen as fast as possible, ideally within the first day after packaging, before the mould become visible on the product. Although the oxygen scavenging activity of CSP was reduced by the presence of the bakery products, CSP could still scavenge the oxygen in the headspace very fast (105–190 min) indicating the potential to be used to prolong the mould free shelf life of bakery products.

3.2. Par-baked buns

When par-baked buns were packed under normal atmosphere (NA), headspace oxygen concentration decreased rapidly from 21 vol.-% to 17.2 vol.-% after 3 days (Fig. 3a). Accompanying with this decrease, 100% of the packed samples were mouldy after 3 days (Fig. 3b). When modified atmosphere (MA) without CO_2 was used the initial oxygen concentration in the packages decreased from 2 vol.-% to below 0.01 vol.-% within 5 days (Fig. 3a). Simultaneously, mould growth was observed in 50% of the packed samples after 3 days, and in 100% after 4 days (Fig. 3b). It can be concluded that a reduction of initial oxygen

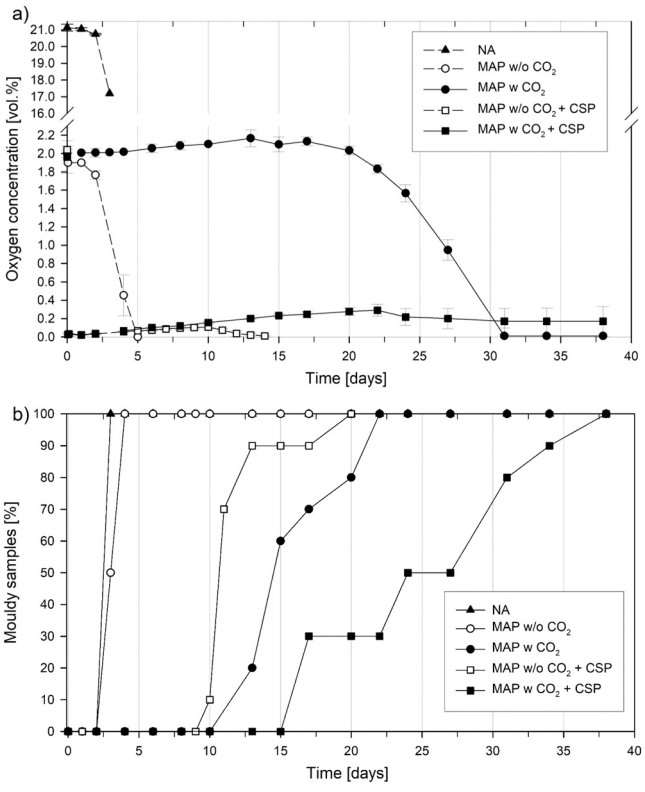

Fig. 3. Change in headspace oxygen concentration of par-baked buns (a) and growth of spoilage moulds on par-baked buns (b) packed under normal atmosphere (NA), modified atmosphere with 93 vol.-% N_2, 5 vol.-% H_2, 2 vol.-%O_2 (MAP w/o CO_2) or modified atmosphere with 93 vol.-% CO_2, 5 vol.-% H_2, 2 vol.-%O_2 (MAP w CO_2) with and without the catalytic system based on palladium (CSP) for 38 days at 25 °C. Mean values ± standard deviation (a: n = 3, b: n = 10).

concentration from 21 vol.-% to 2 vol.-% was insufficient to retard mould growth for par-baked buns. In MA packages with CO_2, the oxygen concentration slightly increased from 2 vol.-% to 2.3 vol.-% during the first 13 days and stagnated afterwards until day 20 (Fig. 3a). Afterwards the oxygen concentration in the headspace decreased fast to below 0.01 vol.-% by day 31 due to microbial spoilage. After 13 days, 20% of the par-baked bun samples exhibited mould development and after 22 days mould was visually detected in all samples (Fig. 3b). It has to be mentioned that compared to MA without CO_2 the mycelium of *A. niger* was extremely weakened in CO_2 atmosphere, which made optical detection more difficult, since a certain size of mycelium is necessary for this. Comparable results with a weakened mycelium of spoilage moulds on wheat and rye bread was reported by Suhr and Nielsen (2005). To sum up, by the use of CO_2 in the MA mould development could be retarded in par-baked buns until day 13 but not completely inhibited.

When CSP was used in combination with MA, the initial oxygen in the headspace was removed within 190 min (Fig. 2). However, in par-baked buns samples packed with CSP under MA without CO_2 the headspace oxygen concentration started to increase after 2 days and reached a maximum of 0.11 vol.-% after 10 days (Fig. 3a). After 14 days, the oxygen concentration decreased to below 0.01 vol.-%. The increase in oxygen concentration in the headspace, enabled moulds to grow and the first mouldy sample was detected after 10 days (Fig. 3b). At day 11, 70% of the samples were mouldy and after 20 days, all par-baked bun exhibited mould development.

Similar to par-baked bun samples packed with CSP under MA without CO_2, the headspace oxygen concentration in the samples packed with CSP but under MA with CO_2 also started to increase after 2 days, but reached a higher value of 0.29 vol.-% after 22 days due to the retardation of the mould growth caused by the presence of CO_2 (Fig. 3a). First mould was visually detected after 17 days (Fig. 3b). After 24 days, 50% of the samples and after 38 days, all samples were mouldy. These results confirm the findings of Suhr and Nielsen (2005), that typical bakery moulds (*Penicillium commune, P. roqueforti* and *Aspergillus flavus*) even grow on wheat bread at levels of 0.03% residual oxygen. Although they did not detect mould growth in MA with 100% CO_2, they found that the spoilage yeast "chalk mould" (*Endomyces fibuliger*) even grew in the presence of an oxygen absorber independent of the CO_2 concentration.

3.3. Toast bread slices

Similar results were obtained with packed toast bread slices compared to par-baked buns, which is also made from wheat. In normal atmosphere (NA) packed toast bread samples, headspace oxygen concentration rapidly decreased after 1 day due to microbial activity (Fig. 4a). After 2 days, 50% of the samples and after 3 days all packed toast bread slices were mouldy (Fig. 4b). In the samples packed under modified atmosphere (MA) without CO_2, after 1 day, the oxygen concentration started to decrease from 2 vol.-% and reached to below 0.01 vol.-% at day 3 (Fig. 4a). In line with this decrease, visual microbial spoilage was detected in 10% of the packed samples after 2 days and in 100% after 3 days (Fig. 4b). In samples packed under MA with CO_2, oxygen concentration increased from 2 vol.-% to 2.5 vol.-% during 22 days and then decreased to below 0.01 vol.-% on day 42 (Fig. 4a). First mould growth was observed after 20 days and after 38 days all samples were mouldy (Fig. 4b).

As shown in Fig. 2 with the use of CSP the headspace oxygen concentration of packed toast bread slices could be decreased from 2 vol.-% to below 0.01 vol.-% within 115 min (Fig. 2). However, after two days the headspace oxygen concentration in toast bread slices packed with CSP in MA without CO_2 started to increase and reached a maximum of 0.14 vol.-% within 6 days (Fig. 4a). Afterwards the oxygen concentration decreased again due to mould growth. After 8 days, microbial growth was detected in 70% of the toast bread samples and after 12 days all samples were mouldy (Fig. 4b). In packages with toast bread slices packed with CSP in MA with CO_2 headspace oxygen concentration started to increase after 4 days and reached a maximum of 0.39 vol.-% after 20 days (Fig. 4a) followed by a decrease afterwards. First, microbial spoilage was detected after 27 days and after 42 days of storage 40% of the samples were mouldy (Fig. 4b).

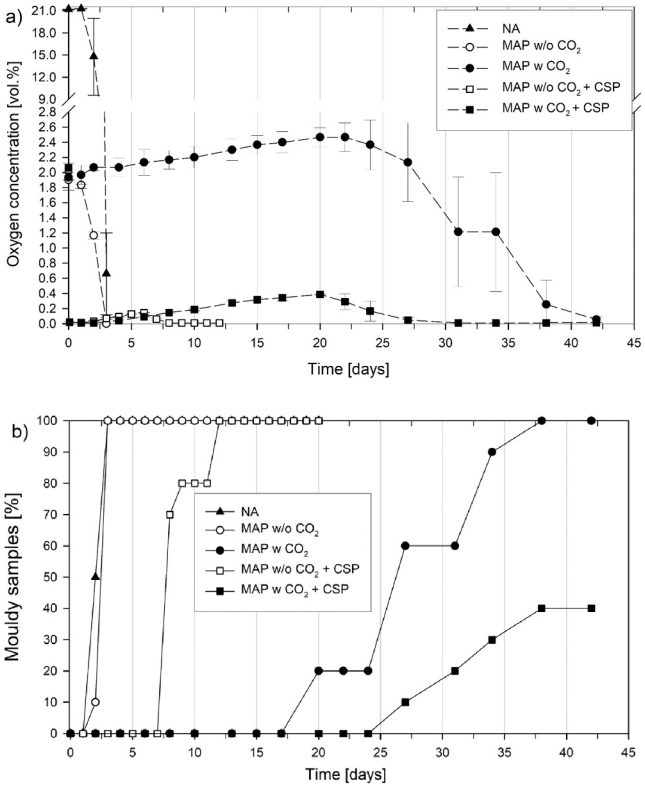

Fig. 4. Change in headspace oxygen concentration of toast bread slices (a) and growth of spoilage moulds on toast bread slices (b) packed under normal atmosphere (NA), modified atmosphere with 93 vol.-% N_2, 5 vol.-% H_2, 2 vol.-% O_2 (MAP w/o CO_2) or modified atmosphere with 93 vol.-% CO_2, 5 vol.-% H_2, 2 vol.-%O_2 (MAP w CO_2) with and without the catalytic system based on palladium (CSP) for 42 days at 25 °C. Mean values ± standard deviation (a: n = 3, b: n = 10).

3.4. Gluten-free bread slices

In normal atmosphere (NA) packed gluten-free bread slices, the oxygen concentration rapidly decreased after 1 day from approximately 21 vol.-% to 15.9 vol.-% (Fig. 5a). Accompanied with this decrease, 100% of the packed samples were mouldy after 2 days (Fig. 5b). Comparable results were obtained for gluten-free bread slices packed under modified atmosphere (MA) without CO_2. After 1 day the headspace oxygen concentration of these packages decreased from 2 vol.-% to 1.0 vol.-% at day 2 (Fig. 5a). Concurrent 90% of the samples were mouldy after 2 days and after 3 days all samples were microbial spoiled (Fig. 5b). In gluten-free bread slices packed under MA with CO_2 the initial oxygen concentration started to decrease after 4 days from 2 vol.-% and reached to below 0.01 vol.-% at day 13, indicating aerobic microbial activity (Fig. 5a). Along with this decrease in headspace oxygen concentration, after 6 days 30%, and after 8 days 100% of the samples exhibited mould growth (Fig. 5b). By the application of CO_2 in the MA the mould free shelf life could be extended from 1 day to at least 4 days.

By the use of the CSP in MA the initial headspace oxygen of 2 vol.-% was removed within 105 min (Fig. 2). After 3 days, an increase in oxygen concentration to a maximum of 0.07 vol.-% at day 5 was detected in gluten-free bread slices packed with CSP under MA without CO_2 (Fig. 5a). In align with this increase, first microbial spoilage was detected after 8 days in 30% of the packed samples (Fig. 5b). Two days

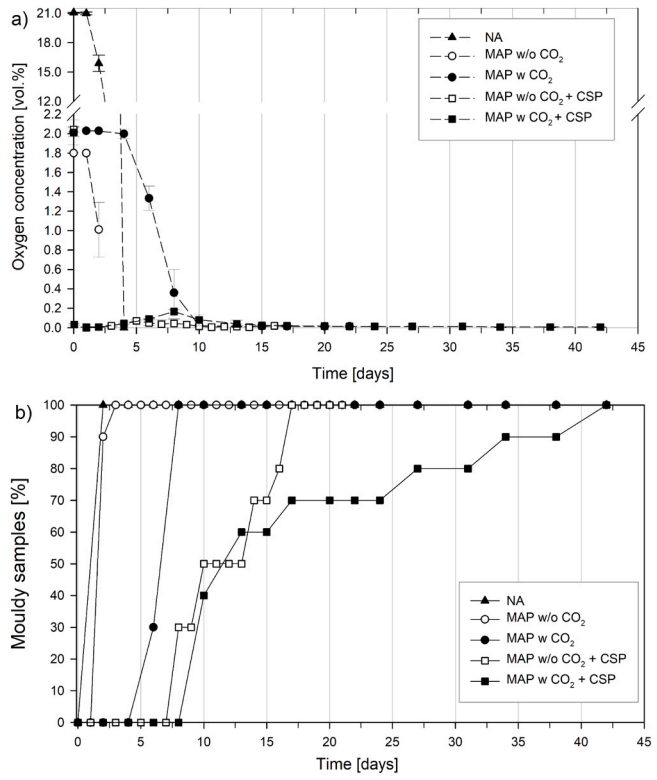

Fig. 5. Change in headspace oxygen concentration of gluten-free bread slices (a) and growth of spoilage moulds on gluten-free bread slices (b) packed under normal atmosphere (NA), modified atmosphere with 93 vol.-% N_2, 5 vol.-% H_2, 2 vol.-%O_2 (MAP w/o CO_2) or modified atmosphere with 93 vol.-% CO_2, 5 vol.-% H_2, 2 vol.-%O_2 (MAP w CO_2) with and without the catalytic system based on palladium (CSP) for 42 days at 25 °C. Mean values ± standard deviation (a: n = 3, b: n = 10).

later, 50% exhibited mould development and after 17 days, all samples were mouldy. In packages with gluten-free bread slices packed with CSP in MA with CO_2 headspace oxygen concentration started to increase after 4 days and reached to a maximum of 0.17 vol% after 8 days. It decreased afterwards to below 0.01 vol% at day 20 (Fig. 5a). First microbial spoilage was detected after 10 days on 40% of the gluten-free bread slices. After 17 days, 70% of the samples were mouldy and after 42 days, on all samples visible mycelium was detected.

4. Discussion

Within this study, it was shown that if no preservatives were used the mould free shelf life of par-baked buns, toast bread slices and gluten-free bread slices packed under normal atmosphere is about 1–2 days (Table 1). The fast microbial spoilage is supported by the high aw-value, which is for all three examined products in the range of 0.95–0.98. This range is optimal for the growth of almost all moulds, bacteria and yeasts (Giannou, Lebesi, & Tzia, 2014). The reduction of the initial headspace oxygen concentration from 21 vol.-% to 2 vol.-% in modified atmosphere (MA) packages without CO_2 was insufficient to prolong the mould free shelf life of the three tested bakery products. The presence of 2 vol.-% oxygen was sufficient for moulds to grow. These results are in line with the findings of Suhr and Nielsen (2005) about spoilage fungi on wheat and rye bread.

Compared to the bakery products packed in normal atmosphere, the use of MA containing CO_2 extended the mould free shelf life by 8–10 days for par-baked buns, 16 – 18 days for toast bread slices and 3 – 4 days for gluten-free bread slices (Table 1). The extension of the mould free shelf life in the presence of CO_2 is due to the fact that CO_2 concentrations higher than 20 vol.-% in the headspace cause the extension of the lag phase and the generation time of spoilage microorganisms. This results in a decrease of the growth rate and consequently delay in the microbial spoilage (Genigeorgis, 1985). Compared to the wheat-based bakery products, gluten-free bread slices are more susceptible to microbial spoilage due to a higher water content (Melini & Melini, 2018), which may explain the short shelf life of the gluten-free bread slices even in the presence of CO_2 in MA. Extension of mould free shelf life through use of modified atmosphere containing CO_2 have also been shown for other bakery products such as unsliced or sliced wheat-based breads and crusty rolls (Degirmencioglu et al., 2011; Smith, Ooraikul, Koersen, Jackson, & Lawrence, 1986; Suhr & Nielsen, 2005). Gutiérrez, Batlle, Andújar, Sánchez, and Nerín (2011) used also MA containing CO_2 (60 vol.-% CO_2 / 40 vol.-% N_2) for gluten free sliced breads and observed a microbial shelf life (mould and yeast) of 7 months. In this study however, the gluten-free sliced breads were not inoculated. The very long shelf life obtained in this study compared to our results could be explained by the absence or low contamination level of moulds in the samples.

The aim of the study was to use the CSP in combination with MA to remove the whole oxygen in the headspace and generate oxygen free environment in the packaging to prevent mould growth in bakery products. By use of the CSP the initial oxygen concentration in all packed bakery products could be successfully removed within a maximum of 190 min and an oxygen free environment was achieved. However, in all bakery products packed headspace oxygen concentration started to increase again after 2–4 days and reached a maximum of 0.11, 0.14, and 0.07 vol.-% (MA without CO_2) and 0.29, 0.39, 0.17 vol.-% (MA with CO_2) for par-baked buns, toast bread and gluten-free bread slices, respectively. This increase in oxygen concentration resulted in mould growth in all products.

Measurements with empty packages without puncturing showed that only 0.065%, 0.025% and 0.020% of the oxygen (MA without CO_2) accumulated in the headspace is due to the permeation of oxygen through the packaging materials for par-baked buns, toast bread and gluten-free bread slices, respectively. For samples packed under MA with CO_2 part of the oxygen accumulated in the headspace due to the permeation through packaging have been similar (0.127%, 0.080% and 0.034%). Additionally, oxygen may have entered the package through the septum-sealed inoculation spot in the lidding film, which may have increased the amount of O_2 permeated through the packaging. Furthermore, the increase in headspace oxygen concentration in bakery packages can be due to the release of oxygen trapped in the par-baked buns, toast bread slices or gluten-free bread slices, into the headspace after the packaging process. It is likely that even more oxygen would

Table 1
Overview mould free shelf life (MFSL) and shelf life extension of par-baked buns, toast bread slices and gluten-free bread slices packed under normal atmosphere (NA), modified atmosphere (MA) without CO_2 (93vol.-% N_2, 5vol.-% H_2, 2vol.-%O_2) or modified atmosphere with CO_2 (93vol.-% CO_2, 5vol.-% H_2, 2vol.-%O_2) with and without the catalytic system based on palladium (CSP). (n = 10), * Shelf life extension due to the use of CSP.

	NA	Modified atmosphere without CO_2			Modified atmosphere with CO_2		
	MFSL	MFSL MA	MFSL MA + CSP	Shelf life extension*	MFSL MA	MFSL MA + CSP	Shelf life extension*
Par-baked buns	2 d	2 d	9 d	+ 7 d	10 – 12 d	15 – 16 d	3 – 6 d
Toast bread slices	1 d	1 d	7 d	+ 6 d	17 – 19 d	24 – 26 d	5 – 9 d
Gluten-free bread slices	1 d	1 d	7 d	+ 6 d	4 – 5 d	8 – 9 d	3 – 5 d

have been accumulated in the headspace, but it has been consumed simultaneously by the moulds growing on the products, which have not been yet visible. This may also explain why higher oxygen concentrations in the headspace were observed when CO_2 was used as a part of the MA. Since CO_2 reduced the growth of moulds, less oxygen was consumed by the moulds during this time. It could be also that part of the oxygen that was diffusing out from the bakery products or permeated through the packaging was initially scavenged by CSP until no more hydrogen was available for the catalytic reaction.

Finally, the application of the CSP in MA without CO_2 extended the mould free shelf life by 7 days for par-baked buns and by 6 days for toast bread and gluten-free bread slices (Table 1) by reducing the initial headspace oxygen. When CSP was combined with MA containing CO_2 the mould free shelf life was further extended by 3 – 6 days for par-baked buns, 5 – 9 days for toast bread slices and 3 – 5 days for toast bread slices. In general, it must be considered, that by the elimination of the headspace oxygen by the CSP, the mould growth could be prevented until the O_2 increased again. However, spoilage yeasts (not the focus of this study) could still lead to spoilage of bakery products even under oxygen exclusion, therefore it is important to use additional CO_2 in the MA to inhibit their growth.

5. Conclusion

It has been demonstrated that a reduction of initial oxygen concentration in the headspace from 21 vol.-% to 2 vol.-% is insufficient to extend the mould free shelf life of the studied bakery products. Use of CO_2 in the modified atmosphere clearly retarded the mould development due to its micro-biostatic effect of CO_2. By the use of CO_2 in the modified atmosphere (MA) mould free shelf life was extended to 10–12 days for par-baked buns, to 17 – 19 days for toast bread slices and to 4–5 days for gluten-free bread slices. The use of the CSP enabled a fast reduction in headspace oxygen concentration of all tested bakery products to below 0.01 vol.-%. However, the diffusion of oxygen from bakery products and permeation of oxygen through the packaging into the headspace of the packaging during the storage enabled the growth of moulds and therefore limited the extension of mould free shelf life of the bakery products. Nevertheless, with the application of the CSP, the mould free shelf life of the tested bakery products could be further extended between 3 and 9 days compared to MA without CSP, independent of the CO_2 concentration. To achieve a greater extension of mould free shelf life for packed bakery products not only the oxygen initially present in the package but also the oxygen coming from the product during storage as well as diffusing through the packaging material should be removed. To achieve this, the CSP could be combined with another oxygen scavenger systems that has a long term oxygen absorption capacity. Efficient removal of oxygen from packaging of bakery products will enable to get rid of use of antimicrobial preservatives and help developing clean label products. But the higher costs associated with the use of two oxygen scavengers must also be considered.

CRediT authorship contribution statement

Nadine Rüegg: Methodology, Investigation, Visualization, Project administration, Writing – original draft and review. **Bettina Röcker**: Investigation, Writing – original draft. **Selçuk Yildirim**: Conceptualization, Writing – review & editing, Supervision, Funding acquisition.

Acknowledgement

This study was funded by Innosuisse (Swiss Innovation Agency) Project No.: 14665.1 PFLS-LS.

References

Axel, C., Zannini, E., & Arendt, E. K. (2017). Mold spoilage of bread and its biopreservation: A review of current strategies for bread shelf life extension. *Critical Reviews in Food Science and Nutrition, 57*(16), 3528–3542. https://doi.org/10.1080/10408398.2016.1147417

Church, N. (1994). Developments in modified-atmosphere packaging and related technologies. *Trends in Food Science & Technology, 5*(11), 345–352. https://doi.org/10.1016/0924-2244(94)90211-9

Deák, T. (2007). *Handbook of food spoilage yeasts* (2nd ed). Boca Raton: CRC Press.

Degirmencioglu, N., Göcmen, D., Inkaya, A. N., Aydin, E., Guldas, M., & Gonenc, S. (2011). Influence of modified atmosphere packaging and potassium sorbate on microbiological characteristics of sliced bread. *Journal of Food Science and Technology, 48*(2), 236–241. https://doi.org/10.1007/s13197-010-0156-4

Dey, A., & Neogi, S. (2019). Oxygen scavengers for food packaging applications: A review. *Trends in Food Science & Technology, 90*, 26–34. https://doi.org/10.1016/j.tifs.2019.05.013

Faas, N., Röcker, B., Smrke, S., Yeretzian, C., & Yildirim, S. (2020). Prevention of lipid oxidation in linseed oil using a palladium-based oxygen scavenging film. *Food Packaging and Shelf Life, 24*, Article 100488. https://doi.org/10.1016/j.fpsl.2020.100488

Galić, K., Curić, D., & Gabrić, D. (2009). Shelf life of packaged bakery goods - A review. *Critical Reviews in Food Science and Nutrition, 49*(5), 405–426. https://doi.org/10.1080/10408390802067878

Genigeorgis, C. A. (1985). Microbial and safety implications of the use of modified atmospheres to extend the storage life of fresh meat and fish. *International Journal of Food Microbiology, 1*(5), 237–251. https://doi.org/10.1016/0168-1605(85)90016-9

Giannou, V., Lebesi, D., & Tzia, C. (2014). Packaging and shelf-life prediction of bakery products. In W. Zhou, Y. H. Hui., I. De Leyn, M. A. Pagani, C. M. Rosell, J. D. Selman, & N. Therdthai (Eds.), *Bakery products science and technology* (pp. 355–371). Wiley Blackwell.

Gibis, D., & Rieblinger, K. (2011). Oxygen scavenging films for food application. *Procedia Food Science, 1*(0), 229–234. https://doi.org/10.1016/j.profoo.2011.09.036

Gutiérrez, L., Batlle, R., Andújar, S., Sánchez, C., & Nerín, C. (2011). Evaluation of antimicrobial active packaging to increase shelf life of gluten-free sliced bread. *Packaging Technology and Science, 24*(8), 485–494. https://doi.org/10.1002/pts.956

Haasum, I., & Nielsen, P. V. (1998). Ecophysiological characterization of common food-borne fungi in relation to pH and water activity under various atmospheric compositions. *Journal of Applied Microbiology, 84*(3), 451–460. https://doi.org/10.1046/j.1365-2672.1998.00368.x

Hutter, S., Rüegg, N., & Yildirim, S. (2016). Use of palladium based oxygen scavenger to prevent discoloration of ham. *Food Packaging and Shelf Life, 8*, 56–62. https://doi.org/10.1016/j.fpsl.2016.02.004

Legan, J. D., & Voysey, P. A. (1991). Yeast spoilage of bakery products and ingredients. *Journal of Apllied Bacteriology, 70*, 361–371. https://doi.org/10.1111/j.1365-2672.1991.tb02950.x

Lohwasser, W., & Wanner, T. (2005). Patent EP 1917139 B1 20120516: Composite system, used as packaging foil, bag foil or partially applied single foil, comprises substrate foil thin film and catalyst for reduction of oxygen.

Melini, V., & Melini, F. (2018). Strategies to extend bread and GF bread shelf-life: From sourdough to antimicrobial active packaging and nanotechnology. *Fermentation, 4*(1), 9. https://doi.org/10.3390/fermentation4010009

Nielsen, P. V., & Rios, R. (2000). Inhibition of fungal growth on bread by volatile components from spices and herbs, and the possible application in active packaging, with special emphasis on mustard essential oil. *International Journal of Food Microbiology, 60*, 219–229. https://doi.org/10.1016/S0168-1605(00)00343-3

Nyberg, C., & Tengstål, C. G. (1984). Adsorption and reaction of water, oxygen, and hydrogen on Pd(100): Identification of adsorbed hydroxyl and implications for the catalytic H2–O2 reaction. *The Journal of Chemical Physics, 80*(7), 3463–3468. https://doi.org/10.1063/1.447102

Pereira de Abreu, D. A., Cruz, J. M., & Paseiro Losada, P. (2012). Active and intelligent packaging for the food industry. *Food Reviews International, 28*(2), 146–187. https://doi.org/10.1080/87559129.2011.595022

Pettersen, M. K., Gällstedt, M., & Eie, T. (2004). Oxygen barrier properties of thermoformed trays manufactured with different drawing methods and drawing depths. *Packaging Technology and Science, 17*, 43–52. https://doi.org/10.1002/pts.642

Restuccia, D., Spizzirri, U. G., Parisi, O. I., Cirillo, G., Curcio, M., Iemma, F., … Picci, N. (2010). New EU regulation aspects and global market of active and intelligent packaging for food industry applications. *Food Control, 21*(11), 1425–1435. https://doi.org/10.1016/j.foodcont.2010.04.028

Röcker, B., Rüegg, N., Glöss, A. N., Yeretzian, C., & Yildirim, S. (2017). Inactivation of palladium-based oxygen scavenger system by volatile sulfur compounds present in the headspace of packaged food. *Packaging Technology and Science, 30*(8), 427–442. https://doi.org/10.1002/pts.2220

Rodriguez, M., Medina, L. M., & Jordano, R. (2000). Effect of modified atmosphere packaging on the shelf life of sliced wheat flour bread. *Nahrung, 44*(4), 247–252. https://doi.org/10.1002/1521-3803(20000701)44:4%3C247::AID-FOOD247%3E3.0.CO;2-I

Sängerlaub, S., Witzgall, S., Müller, K., Wiegert, T., & Pecyna, M. J. (2021). Palladium-based oxygen scavenger for food packaging: Choosing optimal hydrogen partial pressure. *Food Packaging and Shelf Life, 28*, Article 100666. https://doi.org/10.1016/j.fpsl.2021.100666

Seiler, D. A. L. (1989). In A. L. Brody (Ed.), *Controlled/modified atmosphere/vacuum packaging of foods MAP of bakery products* (pp. 119–133). Trumban: Food & Nutrition Press, Inc.

Smith, J. P., Daifas, D. P., El-Khoury, W., Koukoutsis, J., & El-Khoury, A. (2004). Shelf life and safety concerns of bakery products - A review. *Critical Reviews in Food Science and Nutrition, 44*, 19–55. https://doi.org/10.1080/10408690490263774

Smith, J. P., Ooraikul, B., Koersen, W. J., Jackson, E. D., & Lawrence, R. A. (1986). Novel approach to oxygen control in modified atmosphere packaging of bakery products. *Food Microbiology, 3*(4), 315–320. https://doi.org/10.1016/0740-0020(86)90015-8

Suhr, K. I., & Nielsen, P. V. (2005). Inhibition of fungal growth on wheat and rye bread by modified atmosphere packaging and active packaging using volatile mustard essential oil. *Journal of Food Science, 70*(1), M37–M44. https://doi.org/10.1111/j.1365-2621.2005.tb09044.x

Tabak, H. H., & Cooke, W. B. (1968). The effects of gaseous environments on the growth and metabolism of fungi. *Botanical Review, 34*(2), 126–252. https://doi.org/10.1007/BF02872605

Upasen, S., & Wattanachai, P. (2018). Packaging to prolong shelf life of preservative-free white bread. *Heliyon, 4*(9), Article e00802. https://doi.org/10.1016/j.heliyon.2018.e00802

Vilela, C., Kurek, M., Hayouka, Z., Röcker, B., Yildirim, S., Antunes, M. D. C., … Freire, C. S. R. (2018). A concise guide to active agents for active food packaging. *Trends in Food Science & Technology, 80*, 212–222. https://doi.org/10.1016/j.tifs.2018.08.006

Wanner, G. T. (2010). *O2-zehrende und -anzeigende Packstoffe für Lebensmittelverpackungen* (Ph.D. thesis). Technical University of Munich. ⟨https://mediatum.ub.tum.de/doc/972162/⟩.

Yildirim, S., Jammet, J. C., & Lohwasser, W. (2010). Patent EP 2236284: Multi-layer film.

Yildirim, S., Röcker, B., Pettersen, M. K., Nilsen-Nygaard, J., Ayhan, Z., Rutkaite, R., … Coma, V. (2018). Active packaging applications for food. *Comprehensive Reviews in Food Science and Food Safety, 17*(1), 165–199. https://doi.org/10.1111/1541-4337.12322

Yildirim, S., Röcker, B., Rüegg, N., & Lohwasser, W. (2015). Development of palladium-based oxygen scavenger: Optimization of substrate and palladium layer thickness. *Packaging Technology and Science, 28*(8), 710–718. https://doi.org/10.1002/pts.2134